International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 08-10-2021, Accepted: 23-10-2021, Published: 09-11-2021
Volume 6, Issue 6, 2021, Page No. 29-34

Biosynthesis of silver nanoparticles from *Annona squamosa* L. and evaluation of its insecticidal activity

M Gomathi, G Maheswari
Department of Zoology, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, Tamil Nadu, India

Abstract
Nanotechnology is one of the most promising advanced approaches for pest control in recent years. The present investigation was carried out in the laboratory to evaluate the insecticidal activity of Annona AgNPs particles against the insect pest of green gram (*Vigna radiata*). Green biosynthesis of silver nanoparticles (AgNPs) was prepared using *Annona squamosa* leaf extract. The synthesized Annona AgNPs were characterized using UV-vis spectroscopy, FT-IR, TEM, and X-ray diffraction analysis. The phenolic compounds in the aqueous leaves extract pave the way for the possible reduction of silver to nano-silver. XRD- profile of biosynthesized AgNPs revealed that the average size of particle size was 22 ± 6 nm. UV-Vis spectral analysis of Annona AgNPs showed an absorption peak at 315nm. The spectral domine of colloidal solution with broader electronic adsorption bands reveals the occurrence of small spherical silver nanoparticles. FTIR Spectroscopic analysis revealed strong peaks at 3973.36, 3950.22, 3379.29, 2931.80, 2376.30, 2330.01, 1604.77, 1419.61, 1280.73, 107.42 and 624.94 cm-1. The strong bands at 624.94cm-1 are attributed to the stretching vibration of the C-CL functional group of alkyl halide. The peaks at 1072corresponds to C-N stretching vibration aliphatic amines, and weaker bands at 1280.73cm-1 represent the in-plane bending vibration of the C-N stretching vibration of aromatic amines. The absorption peaks at 1419.61 and 160.77cm-1 are assigned to the bending vibration of N-H amine. The smaller peak at 2931.80 and broader band at 3379.29 were identified as C-H stretching of alkanes and stretching vibration of N-H amine, respectively. TEM analysis shows that the Annona AgNPs particles were spherical metal particles with an average size of 162nm. The contact bioassay method was followed to assess the insecticidal activity of *Annona* AgNPs against the pulse beetle, *C. analis*. The results revealed that cent percent death was observed on VII day in 100% concentration and showed its effectiveness. The concentration of 60 and 80% were equally effective in arresting *C. analis* population in green gram. From the study, it was obvious that the *Annona* AgNPs particles were the most powerful, safe, non – toxic natural insecticides that can be used to manage bruchid infestation during storage.

Keywords: biosynthesis, *Annona squamosa* L., insecticidal activity, nanotechnology

Introduction
Nanomaterials have a wide range of applicability in improving human life and its environment. The first relation between human life and nanoscale was developed naturally in Ayurveda, which is a 5000-year-old Indian system of medicine (Dubey *et al.*, 2010) [3]. The use of plants in the synthesis of nanoparticles is quite novel leading to truly green chemistry, which provides advancement over chemical and physical methods as it is cost-effective and environment-friendly, suitable for large-scale synthesis. This method does not require high pressure, energy, temperature, and toxic chemicals (Maggy and Gordon, 2006; Bhainsa and Souza, 2006) [7, 2]. The silver nanoparticles have various other vital applications. From time immemorial, silver has been known to have a disinfecting effect and used in traditional medicine. Silver nanoparticles (SNPs) are reported nontoxic to humans and most effective against bacteria, viruses, and other eukaryotic microorganisms at low concentrations and without side effects (Jeong *et al*, 2005) [4].
The major advantage of utilizing plant extracts for silver nanoparticle synthesis is that the plants are locally available, safe, nontoxic, and have many metabolites. These metabolites aid in reducing silver ions, which are quicker than microbes in the synthesis of AgNPs. The main mechanism considered for the process is a plant-assisted reduction due to phytochemicals. The main phytochemicals involved are terpenoids, flavones, ketones, aldehydes, amides, and carboxylic acids. Flavones, organic acids, and quinones are water-soluble phytochemicals that are responsible for the immediate reduction of the ions (Prabhu and Poulose, 2012) [8].
The use of Annona to synthesize AgNPs was reported by many researchers (Senthamilselvi *et al.*, 2013; Vivek *et al.*, 2012; Kumar *et al*., 2012) [10, 11, 5] However, the activity of *Annona* AgNPs against Pulse beetle was not reported. Hence, an attempt was made in the laboratory to evaluate the insecticidal activity of Annona AgNPs against Pulse beetle, *Callosobrucus analis*.

Materials and Methods
An investigation was carried out in the laboratory to synthesize green nanoparticles using *Annona squamosal* L. and to evaluate its insecticidal activity against the pulse beetle, *Callosobrucus analis.*

Collection of plant material
The leaves of *Annona squamosa* (L.) were collected in and

around Coimbatore District, Tamilnadu, India. The plant was taxonomically identified and authenticated by the Botanical Survey of India (BSI), Coimbatore, Tamilnadu, India, and voucher specimen number BSI/SRC/5/23/2013-14/TECH. 2033 is deposited in our laboratory for further references.

Extraction procedure

Annona Squamosa (L.) leaves were washed with distilled water to remove dirt and soil and cut into fine pieces. The cut pieces were boiled at 60oc for 20 minutes to obtain the aqueous extract. The suspension was filtered using Whatman No.1 filter paper, and the filtrate was used to synthesize Annona silver nanoparticles (AgNPs).

Biosynthesis of *Annona squamosa* AgNPs

For biosynthesis of AgNPs, a blend of 100 ml 1mm silver nitrate and 100 ml of aqueous *A. Squamosa* leaf broth was heated at 60oc until no further colour change. The colour intensity of the colloidal solution gradually increased by the effect of temperature. Precisely after 20 minutes, there is no change in colour indicating the completion of nanoparticle formation. The AgNPs suspension was allowed to dry using Nano Spray Dryer B90 at Curie Laboratory, Avinashilingam University, and the powder obtained was used for further analysis

Characterization of AgNPs

The biosynthesized AgNPs were well characterized by the following techniques.

The optical properties of AgNPS were monitored by a Shimadzu UV-2450 Spectrophotometer at a resolution of 1nm, between 200-800nm using 10nm optical path- length-quartz cuvettes. The molecule's functional group us phytoconstituents present in the AgNPs were determined by Fourier-Transform-Infrared (FTIR). FTIR analysis was performed using spectrum RX-1 instrument in diffuse reflectance mode operated at a resolution of 4 cm^{-1} of the wavelength of about 4000-400cm-1 using KBr pellets. Morphology of nanoparticles was examined by Transmission Electron Microscope (TEM). XRD measurements were carried out using a Philips 86 „X" pert pro-X-ray diffractometer by preparing a thin film on a glass substrate. The biosynthesized nanoparticles' surface charge (stability) was analyzed with Malvern Zetasizer (Nano ZS90, UK) instrument.

Insecticidal activity of Annona *Squamosa* (L) AgNPs

Adults of *C. analis* were reared at 30±10c and 65± 5% RH in a BOD incubator. Insects were obtained from cultures maintained in our laboratory for at least 5 years with no history of exposure to insecticides. Freshly emerged adults from the culture were used for bioassay studies. The influence of Annona AgNPs on the adults of *C. analis* was determined by Contact Toxicity Assay at 5 different doses (20%, 40%, 60%, 80%, and 100%). The experiment was laid out in a Completely Randomized Design (CRD) with 5 treatments and 3 replication.

Different doses of Annona AgNPs were sprayed individually on Petri plates and allowed to dry for 3 hours. In each Petri plate 5 pairs of *C. analis* were introduced. Petri plate without Annona AgNPs was considered as control with 5 pairs of insects. Daily observation of mortality of insects was recorded from 1-7 days and the results were tabulated.

Statistical analysis

The data on the percentage mortality of *Callosobrucus analis* were subjected to two-way analysis of variance and the mean values obtained were analysed using Duncan's Multiple Range Test (DMRT).

Result and Discussion

The biosynthesis of silver nanoparticles has attracted many researchers due to their applicability in various fields. However, the utilization of AgNPs made from plant products as insecticides is meager. In addition, the literature survey revealed the green AgNPs are not tested against the notorious pulse beetle, *Callsobruchus analis*. The present study was initiated to synthesize AgNPs using *Annona squamosa* as a reducing agent and evaluate its insecticidal activity against the pulse beetle, *Callsobruchus analis* in green gram, *Vigna radiata*.

Characterization of AgNPS

XRD analysis

The green synthesized Annona AgNPs were subjected to various characterization studies. The structural properties of the prepared nanoparticles were analysed by Seifert X-ray diffracto meter. The XRD profile of biosynthesized AgNPs was depicted in Fig 1. From the XRD profile, four distinct diffraction peaks at 2θ =27.8o, 32.2o, 38.2o, and 46.1o were perceived with the size of 20 nm, 31nm, 21nm, and 16nm respectively. The average size of the synthesized particle was calculated as 22 ± 6 nm. Crystalline structure of AgNPs can be calculated using Debye-Scherer's formula, D = kλ/βcosθ, 0Whereas D is the crystal size of AgNPs, λ is the wavelength of the X-ray source used (1.541Ao), β is the full width at half maximum of the diffraction peak, K is the constant of the Debye-Scherer equation with the value from 0.9 to 1 and θ is the Bragg angle. (22). The four different structural peaks present in the XRD pattern and crystalline size suggest that the biosynthesized Annona AgNPs were crystalline.

The peaks 27, 77, 32, 22, 38, 16 and 46.08 were due to the organic compounds present in the extract. The exact mechanism by which the formation of Ag_2O was explained by Senthamilselvi *et al.*, 2013 [10] who reported that Ag_2O formation may be due to coupling reaction with phenolic compounds. A similar kind of result in the biosynthesis of silver nanoparticles using seaweed, *Ulva lactuca* was reported by Kumar *et al.*, 2012 [5].

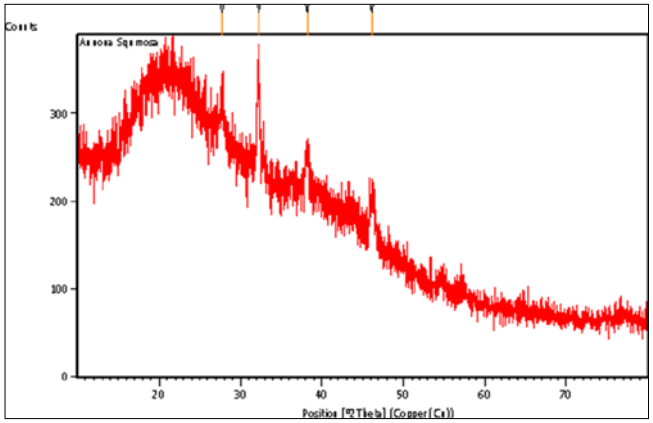

Fig 1: X-RAY diffraction patterns of *Annona* silver nanoparticle

UV-Vis spectral analysis

The formation of silver nanoparticles was confirmed by UV-Vis-spectroscopy analysis. The aqueous extract of *Annona* was dark yellow, which was changed to black when 1Mm AgNO3 was added and kept in a water bath at 60°c for 20-30minutes. However, no color change was observed for the 1Mm AgNO3 control solution. UV-Vis spectral analysis of the control and biosynthesis of silver nanoparticles from *Annona* leaf extract was shown in Figures 2 and 3.

The photo reduction of Ag+ in an aqueous solution was monitored over 30 minutes. It was clear from Fig 4 that the absorption of silver appears between 300-400 nm. In comparison, no such peak was observed for the control. (Fig.2) Broader peaks signify smaller particle size and reflect the effects of experimental conditions on the nucleation and growth at the crystal nuclei (Becheri *et al.*, 2008).

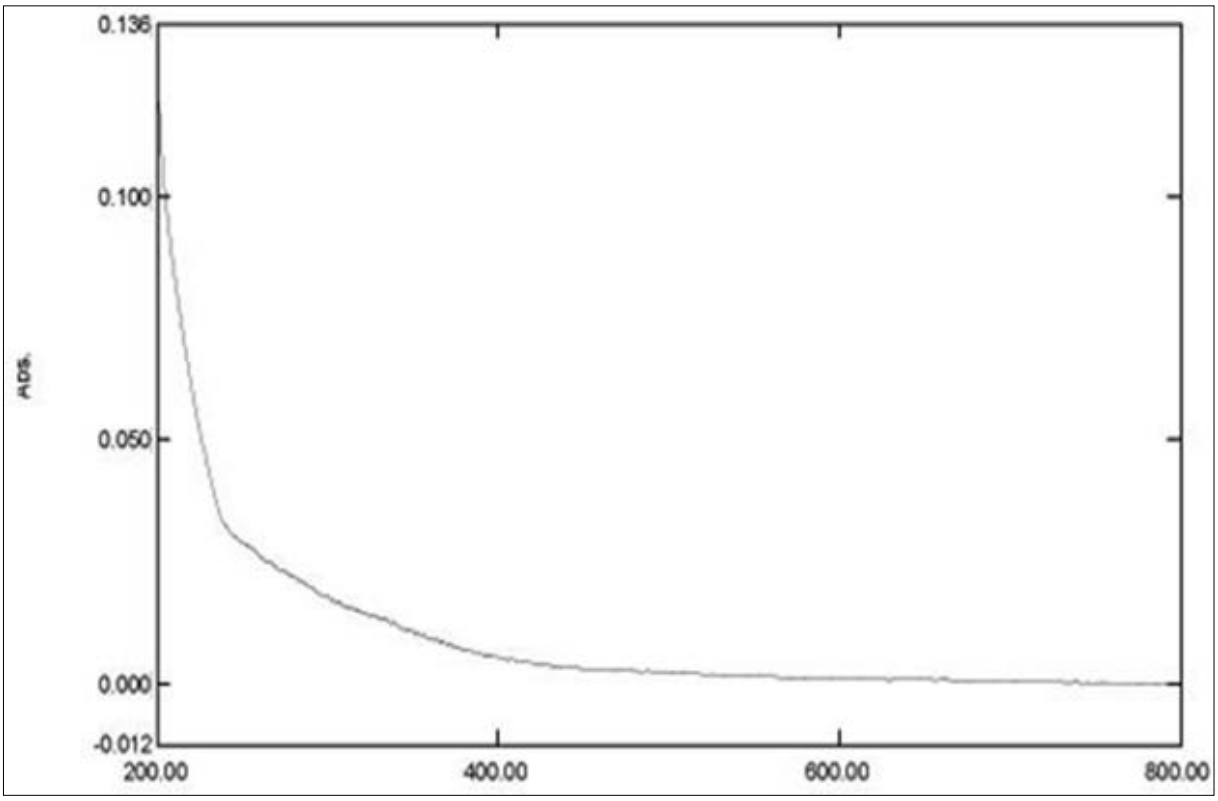

Fig 2: UV-VIS spectroscopical analysis *Annona* of control

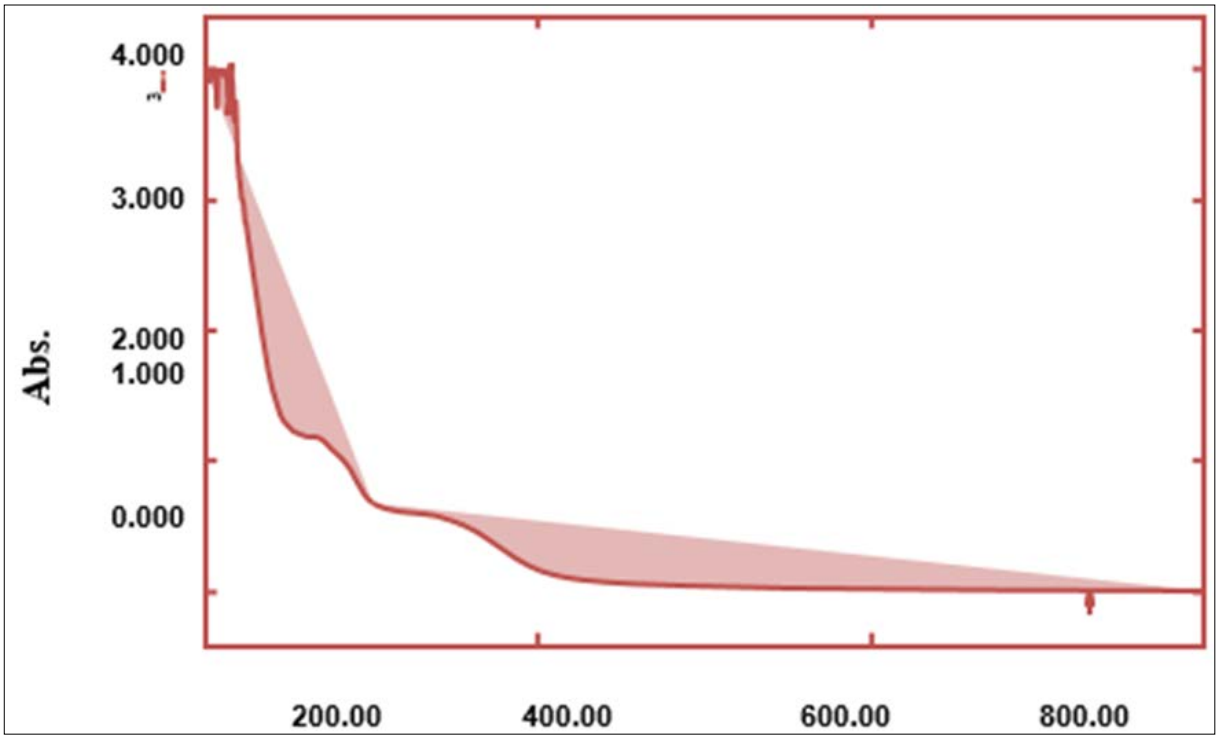

Fig 3: UV-VIS spectroscopical analysis of *Annona* aqueous extract

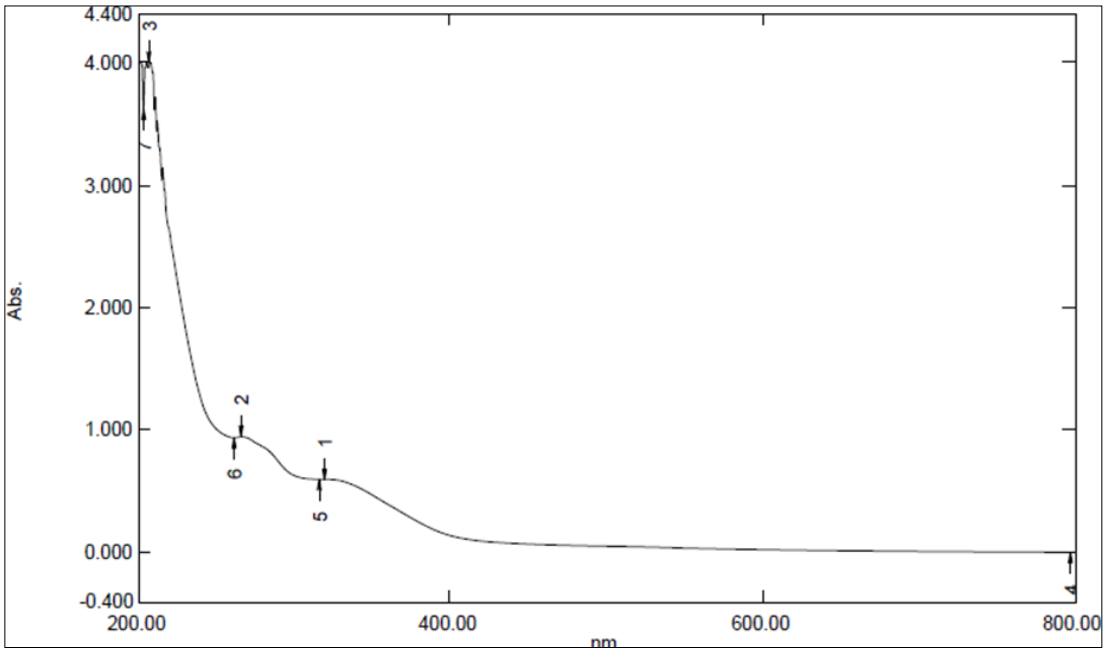

Fig 4: UV-VIS spectroscopical analysis of *Annona* silver

Fourier transform infrared spectroscopy (FTIR)

The information on vibrational and rotational modes of motion of a molecule could be retrieved using Infrared spectroscopy and hence is an essential technique for the identification and characterization of a substance (Lalitha *et al.*,2013) [6].

The FTIR spectroscopy analysis identified the possible biomolecules responsible for reducing Ag++ ions and capping of AgNPs. This figure revealed strong peaks at 3973, 3950, 3379, 2931, 2376, 2330, 1604, 1419, 1280, 1072 and 624.94 cm-1. The strong bands at 624.94cm-1 are attributed to the stretching vibration of the C-CL functional group of alkyl halide. The peaks at 1072 correspond to C-N stretching vibration aliphatic amines, and weaker bands at 1280.73cm-1 represent the in-plane bending vibration of the C-N stretching vibration of aromatic amines. The absorption peaks at 1419.61 and 160.77cm-1 are assigned to the bending vibration of N-H amine. The smaller peak at 2931.80 and broader band at 3379.29 were identified as C-H stretching of alkanes and stretching vibration of N-H amine respectively. These biomolecules were identified as possible stabilizing groups that contributed to the nano structuring of the silver ion and it was evident from the FTIR spectra in Fig 5.

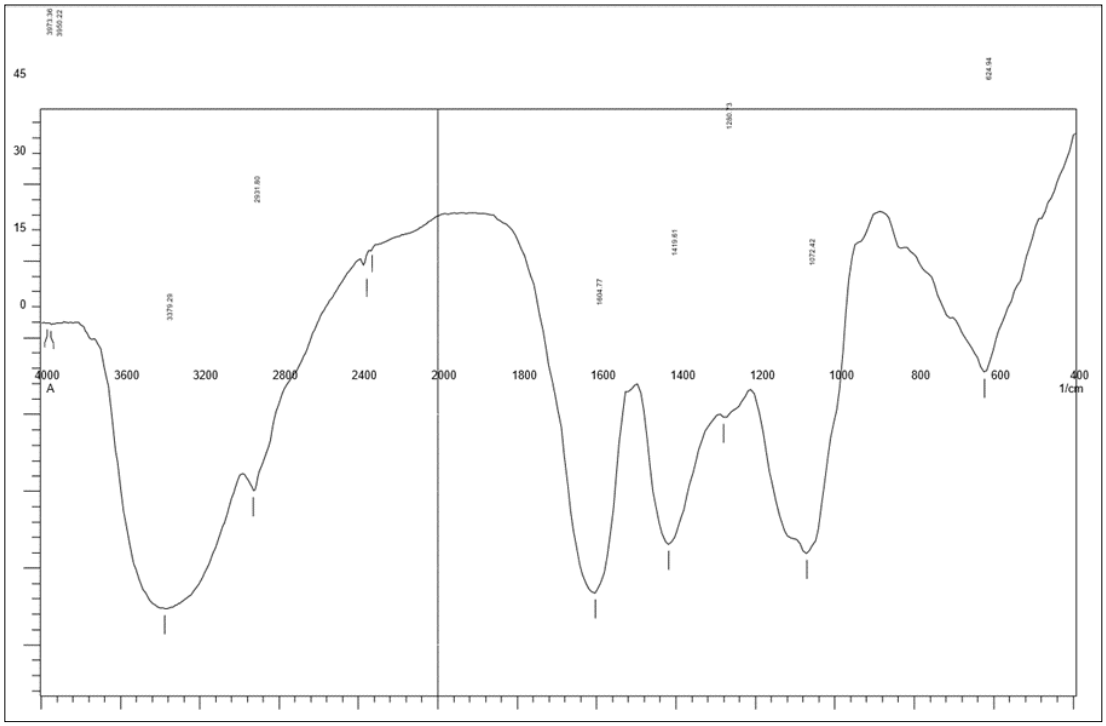

Fig 5: FTIR spectrum of *Annona* silver nanoparticle

TEM analysis

The morphology and particle size of silver nanoparticles were determined by TEM. Fig.6 shows that the Annona AgNPs particles were spherical metal particles with an

average size of 162nm. Based on the excitation of surface Plasmon resonance, 30 minutes is sufficient for the formation of spherical AgNPs. These findings corroborate with Senthamilselvi *et al.*,2013 [10] who reported the spherical shape of Annona AgNPs with an average particle size of 52nm. In accordance with the findings of Vivek *et al.* (2012) [11] and Kumar *et al.*,(2012) [5], who reported that the formation of nanoparticles is rapid depicting narrow size distribution. The particles display inter particular distances with no physical control due to electrostatic repulsive force.

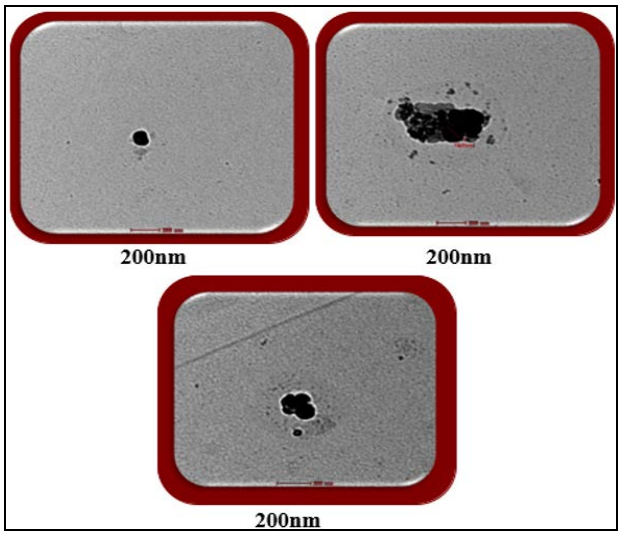

Fig 6: Tem images of annona silver nano particles

Insecticidal activity of annona AgNPs

To assess the insecticidal activity of Annona AgNPs, the Contact Bioassay method was followed with five doses (20, 40, 60, 80, and 100%). Mortality of *Callosobrucus analis* was noticed from the I day onwards. Significant differences in the mortality of insects were observed between treatments and control. Maximum deaths of insects were recorded from the 1st day onwards for 80% (3.3%) and 100% concentration (10%). However, the control and other treatments registered nil mortality on the first day of observation. It was evident from the Table 1 that cent percent death was observed on VII days in 100% concentration and proved its effectiveness. Compared with control (26.6%), aqueous *Annona* extracts and 1Mm AgNO3 registered a higher percentage of mortality on the VII day of observation (56.6%). This was followed by a 60% dose which recorded the mortality percentage of 93.3% on the VII day of observation. This was followed by a 60% dose which recorded the mortality percentage of 93.3 on VII day and 80% with 86.7% on the same day. Moderate insecticidal activity was recorded for 20% and 40% doses and the mortality ranging from 6.7-80%.

This was similar to the findings of Rouhani *et al.*, 2012 [9] who tested the insecticidal effect of silica and silver nanoparticles on *Callosobrucus analis* and revealed that both nanoparticles (silica and silver) were highly effective on adults and larvae with 100% and 83% mortality respectively.

Table 1: Insecticidal activity of *Annona* $AgNP_S$ against *Callosobruchus analis*

Concentration	Treatment							Mean
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	
Control	0.0^a	0.0^a	0.0^a	3.33^a	10^a	16.7^a	26.7^a	8
$AgNO_3$	0.0^b	0.0^b	6.7^b	16.7^b	30^b	43.3^b	56.7^b	21.9
Plant extract	0.0^b	0.0^b	6.7^b	20^b	33.3^b	43.3^b	56.7^b	22.8
AgNPs 20%	0.0^c	0.0^c	6.7^c	23.3^c	40^c	60^c	80^c	30
AgNPs 40%	0.0^c	0.0^c	6.7^c	20^c	43.3^c	66.7^c	76.7^c	30
AgNPs 60%	0.0^d	3.3^d	16.7^d	36.7^d	70^d	86.7^d	93.3^d	43.8
AgNPs 80%	3.3^d	13.3^d	27.3^d	46.7^d	70^d	83.3^d	86.7^d	47.2
AgNPs 100%	10^e	33.3^e	53.3^e	73.3^e	86.7^e	96.7^e	100^e	64.7
Mean	1.6	6.2	15.5	30	47.9	62	72	GM=33.649

Conclusion

Many researchers did not study the impacts of NPs on stored grain pests. The present study is a new attempt to test the efficacy of Annona AgNPs against the Pulse beetle, *Callosobrucus analis*. It was obvious from the observation that the application of Annona AgNPs reduces the density of *Callosobrucus analis* population in green gram. Moreover, Annona AgNPs can be easily removed by conventional milling processes and do not leave any residue on the grain. Therefore, Annona AgNPs possess excellent insecticidal potential against the pulse beetle and can be recommended as insecticide to protect the pulses from bruchid infestation.

Reference

1. Bacheri A, Durr M, Nostra PL, Baglioni P. Synthesis and characterization of zinc oxide Nanoparticles: application to textiles as UV-absobers. J. Nanopart. Res,2008:10:679.
2. Bhainsa KC, Souza SFD. Extracellular biosynthesis of silver nanoparticle using the fungus, *Aspergillus fumigatus.* Colloids Surf B,2006:47:160-164.
3. Dubey SP, Lahtinen M, Särkkä H, Sillanpää M. Bioprospective of *Sorbus aucuparia* leaf extract in development of silver and gold nanocolloids. Colloids surf B Biointerfaces,2010:80:26-33.
4. Jeong SH, Yeo SY, Yi SC. The effect of filler particle size on the antibacterial properties of compounded polymer/silver fibers. J. Mat. Sci,2005:40:5407-5411.
5. Kumar P, Senthamilselvi S, Lakshmipraba A0, Premkumar K, Muthukumaran R. Efficacy of bio-synthesized silver nanoparticles using *Acanthophora spicifera* to encumber biofilm formation. Dig J Nanomat Biostruc,2012:2:511-522.
6. Lalitha A, Subbaiya R, Ponmurugan P. Green synthesis of silver nanoparticles from leaf extract *Azhadirachta indica* and to study its anti-bacterial and antioxidant property. Int. J. Curr. Microbiol. App. Sci,2013:2(6):228-235.
7. Maggy L, Gordon S. Bioaccumulation of gold by sulfate-reducing bacteria cultured in the presence of gold (I)-thiosulfate complex. Acta,2006:70(14):3646-3661.

8. Prabhu S, Poulose EK. Silver Nanoparticles: Mechanism of Antimicrobial Action, Synthesis, Medical Applications, and Toxicity Effects. Prabhu and Poulose International Nano Letters,2012:2:32. http://dx.doi.org/10.1186/2228-5326-2-32
9. Rouhani M, samih MA, kalantari S. Insecticide effect of silver and zinc nanoparticles against *Aphis nerii* boyer de fonscolombe (hemiptera: aphididae). Chilean journal of agricultural research,2012:72(4):590-594.
10. Senthamilselvi S, Kumar P, Prabha AL, Govindaraju M. Green simplistic biosynthesis of anti- bacterial silver nanoparticles using *Annona squamosa* leaf extract. Nano Biomed. Eng,2013:5(2):102-106.
11. Vivek R, Thangam R, Muthuchelian K, Gunasekaran P, Kaveri K, Kannan S. Green biosynthesis of silver nanoparticles from *Annona squamosa* leaf extract and its in vitro cytotoxic effect on MCF-7 cells. Process Biochem,2012:47:2405-2410.